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Original Citation:

Renal adaptation to stress: A possible endothelin release and prostaglandin in the human subject / SERGIO CASTELLANI; ANDREA UNGAR; GIUSEPPE LA CAVA; CLAUDIA CANTINI; CRISTINA STEFANILE; ALBERTO CAMAITI; GIANNI MESSERI; MIRELLA COPPO; BARBARA VALLOTTI; CLAUDIA DI SERIO; ALESSANDRO BROCCHI; GIULIO MASOTTI. - In: JOURNAL OF LABORATORY AND CLINICAL MEDICINE. - ISSN 0022-2143. - ELETTRONICO. - 129:(1997), pp. 462-469.

Availability:

This version is available at: 2158/743925 since:

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Renal adaptation to stress: A possible role of endothelin release and prostaglandin modulation in the human subject

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The aim of this study was to define the neurohumoral response associated with the renal hemodynamic perturbations induced by mental stress acting as an adrenergic stimulus. In 8 healthy women, the effects of mental stress were studied during four consecutive 30-minute periods (baseline, mental stress, recovery I, recovery II). Mental stress induced sympathetic activation as evidenced by increases in blood pressure, heart rate, and plasma norepinephrine level. Effective renal plasma flow (iodine 131-labeled hippurate clearance) decreased only during mental stress (-22% , $p < 0.05$ vs baseline); glomerular filtration rate (iodine 125-labeled iotalamate clearance) remained constant during the entire experiment; the filtration fraction increased significantly during mental stress and recovery I ($+30\%$ and $+22\%$, respectively, $p < 0.02$ for both). Complex neuroendocrine responses were associated with the hemodynamic changes. Urinary excretion of endothelin-1 and 6-keto-PGF_{1 α} increased during mental stress ($+53\%$, $p < 0.01$, and $+20\%$, $p < 0.01$, respectively) and recovery I ($+49\%$ and $+29\%$, respectively, $p < 0.01$ for both). Urinary cyclic guanosine monophosphate rose only during mental stress ($+77\%$, $p < 0.05$), whereas excretion of PGE₂ showed a stepwise increase throughout recovery I and II ($+292\%$, $p < 0.01$, and $+360\%$, $p < 0.001$, respectively). In conclusion, the present experiments demonstrate that renal hemodynamic response induced by mental stress is a complex reaction in which endothelin-1, prostaglandins, and presumably nitric oxide take part. (J Lab Clin Med 1997;129:462-9)

Abbreviations: ANF = atrial natriuretic factor; ANOVA = analysis of variance; cGMP = cyclic guanosine monophosphate; EDTA = ethylenediaminetetraacetic acid; ERPF = effective renal plasma flow; HR = heart rate; GFR = glomerular filtration rate; PRA = plasma renin activity; UPGE₂ = urinary prostaglandin E₂; UPGF_{2 α} = urinary prostaglandin F_{2 α} ; UTXB₂ = urinary thromboxane B₂; U6-keto-PGF_{1 α} = urinary 6-keto-prostaglandin F_{1 α}

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Submitted for publication July 29, 1996; revision submitted Nov. 21, 1996; accepted Nov. 27, 1996.

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0022-2143/97 \$5.00 + 0 5/1/79581

The kidney adapts to stimulation of the sympathetic nervous system with a complex reaction that keeps GFR constant.^{1,2} An important part of this reaction is the activation of a wide array of neurohumoral systems, the roles of which are not fully understood, particularly in terms of an integrated response. The renin-angiotensin and adrenergic nervous systems and renal eicosanoids are known to be involved.³⁻⁶ However, the role of newly discovered substances such as endothelins and nitric

xide in the response of the human kidney to stressful stimuli has not been defined. Endothelin is abundantly synthesized by the endothelial and mesangial cells of the kidney⁷ and causes a sustained and prolonged renal vasoconstriction and a marked decrease in glomerular filtration rate.⁸ Endothelin also interacts with the three systems enumerated above: it can be released by both angiotensin II and adrenergic stimulation^{7,9,10} and, in turn, endothelin stimulates renal eicosanoid release in *in vitro* and animal experiments.^{11,12} In addition, endothelin has been reported to promote the formation of nitric oxide in the kidney.¹³

Both animal and human studies^{14,15} have shown that adrenergic stimulation may lead to a sharp decline in renal blood supply. This decline may be aggravated to the point of causing acute renal failure when it is associated with an underlying renal disease¹⁶ or when there is a derangement of the neurohumoral systems, such as the inhibition of renal eicosanoids during nonsteroidal anti-inflammatory drug administration.^{17,18}

To have some understanding of the possible mechanisms of this shift from vasoconstriction to ischemic damage or renal failure, it is mandatory to first fully understand the physiologic renal response to adrenergic stimulation in its integrated setting of hormonal reactions.

Therefore, the aim of the present study was to examine, in healthy human subjects, the neurohumoral response that parallels renal hemodynamic adjustments evoked by adrenergic stimulation as induced by a well-defined mental stress. In particular, the "classical" vasoactive systems (namely plasma renin activity, catecholamine, and prostaglandins) and the changes in time in endothelin and cGMP as an index of nitric oxide/atrial natriuretic factor activation were evaluated.¹⁹⁻²¹

METHODS

Subjects. Experiments were carried out in 8 healthy nonpregnant female volunteers who were 24 to 40 years of age (mean \pm SD, 31 ± 6) and who had given their informed consent to participate in the study. All subjects were women, given that urinary prostaglandins may have an extrarenal origin in males.²² Volunteers with hypertensive parents were excluded from the study because vascular response to sympathetic stimulation has been found to be altered in these subjects.²³ The volunteers were considered healthy on the basis of medical history, clinical examination, blood chemistry, urinalysis, electrocardiogram, and renal ultrasound evaluation. No subject had taken either aspirin or any other cyclooxygenase-inhibiting drug for 15 days before the beginning of the study; no subject was taking oral contraceptives. All subjects were

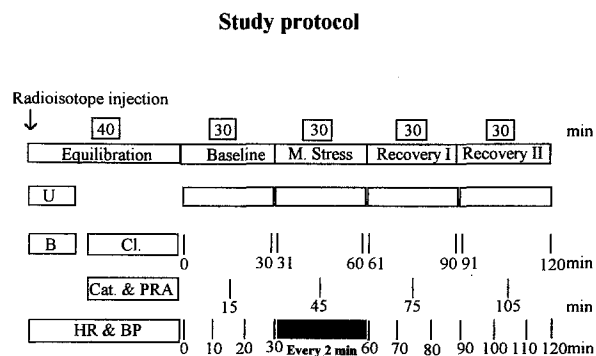


Fig. 1. U, Urine sampling; B Cl., blood sampling for ¹³¹I-labeled hippuran and ¹²⁵I-labeled iothalamate clearances; B Cat & PRA, blood sampling for norepinephrine, epinephrine, and PRA; HR & BP, HR and blood pressure recording.

nonsmokers, had body mass indexes of less than 27, and had plasma renin activity within the normal range.

Study protocol. The study was performed after the sodium balance had been established by a 5-day diet containing 108 mmol sodium chloride and 60 to 80 mmol potassium. Urinary electrolytes were measured in the 3 days before the stimulus so as to ensure that they reached equilibration (urinary sodium = 70 to 90 mmol/24 hours and urinary potassium = 40 to 60 mmol/24 hours). Caffeine- and alcohol-containing beverages were not allowed on the day of investigation. To achieve a constant urinary flow, all subjects were studied under moderate hydration, after having drunk 100 ml of tap water every hour on the hour from 8 AM until 2 PM on the day of the investigation. At noon all subjects had a light meal without any animal protein to avoid renal vasodilation. Experiments were performed with the subjects in the supine position. A catheter was inserted into the urinary bladder to collect urinary samples. At the same time, an 18-gauge polytef catheter needle with a three-way stopcock was also inserted into the antecubital vein to collect blood samples. Each blood sample consisted of a volume of 7 ml of blood, which was immediately placed into ice-chilled test tubes containing EDTA, 10 mmol/L final concentration. A 0.95% NaCl solution was continuously intravenously infused at a rate of 1.6 ml/min to compensate for plasma volume reduction after repeated blood sampling. Soon after catheterization of the urinary bladder, two radioisotopes were injected subcutaneously (iodine 125-labeled iothalamate, 40 μ Ci, and iodine 131-labeled hippuran, 80 μ Ci) for the measurement of renal clearances. A 40-minute equilibration period was needed to achieve constant plasma radioactivity; only after this time had elapsed did the experiment begin. The study consisted of four successive 30-minute experimental periods: baseline determination, mental stress, and two recovery periods (Fig. 1). Blood pressure and HR were measured every 10 minutes during baseline and the two recovery periods and every 2 minutes during the mental stress period. The blood samples for the determination of the cat-

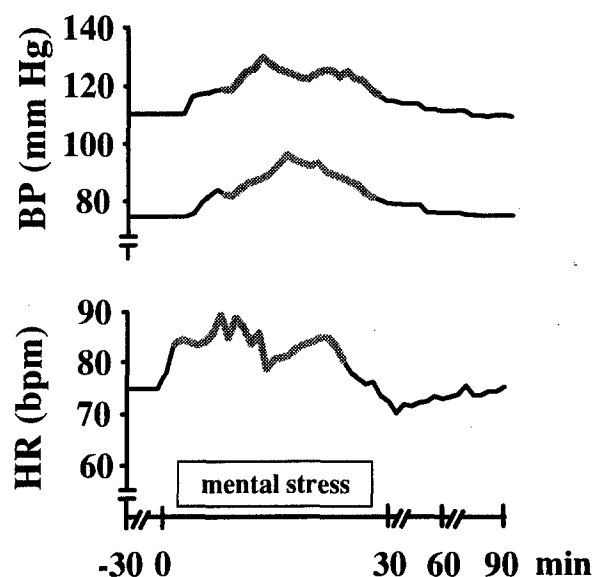


Fig. 2. Effects of mental stress on blood pressure and HR ($n = 8$). Cross-hatch area indicates values significantly different from baseline ($p < 0.001$ for each curve, Duncan test, ANOVA).

echolamines (norepinephrine and epinephrine) and PRA were drawn in the middle of the mental stress period (when the expected arousal reaction should have reached its maximum) and in the middle of both the baseline and recovery periods. Blood samples for renal clearance determinations were drawn at the beginning and at the end of every experimental period. The 30-minute urinary samples for urinary autacoids, renal clearance, and chemical determinations were collected during each experimental period through the urinary bladder catheter.

Preliminary experiments. A preliminary 2-hour experiment in 5 healthy subjects was devoted to verifying the stability and the reproducibility of the measurement of ERPF, GFR, blood pressure, and HR. In this preliminary study, all conditions were the same as in the experimental study except that mental stress was not applied. In this study, blood pressure, HR, ERPF, and GFR were all steady.

Mental stress. Mental stress was induced via the Fair Culture Test, a multiple-choice image questionnaire. This test was chosen over other forms of psychologic stimuli given that it does not require any degree of cultural background, such as the solving of arithmetic problems or the reading of a text. Indeed, the Fair Culture Test can be administered at any age, without any regard to scholastic level.²⁴ The test is made up of 50 questions, subdivided into four sets; each set is preceded by a full explanation and a preliminary test. To elicit a maximal anxiety reaction, the time given for answering all questions is carefully controlled. This is a fundamental feature of the stimulus, given that time dependence is known to elicit significant and increasing anxiety whenever an intellectual performance is urged. All aspects of the testing had to be completed within 30 minutes, according to the study protocol.

Systemic hemodynamic parameters. Arm blood pressure was measured by using a sphygmomanometer; the first Korotkoff sound was taken as an index of systolic blood pressure, and the fifth was taken as an index of diastolic blood pressure. Mean blood pressure was obtained by adding one third of the pulse pressure to the value of the diastolic pressure.²⁵ HR was monitored by electrocardiogram recording.

Renal hemodynamic parameters. ERPF and GFR were measured according to the methods described by Adefuin et al.²⁶ and Ram.²⁷ The only variation in methods consisted in not calculating the mean plasmatic radioactive concentration between the consecutive sampling points but instead making calculations with a trend of the plasma curve within each sampling interval. In our study, ERPF and GFR data were analyzed by fitting a monoexponential curve onto the experimental data. Within this framework, the mean plasmatic radioactive concentration within each interval was calculated as the ratio between the integral of the fitted curve within the time boundaries of the interval of interest and the length of the interval. The fitting procedure was derived from the iterative Nelder-Mead simplex search algorithm²⁸ (Matlab software package; Mathworks, Inc., Natick, Mass; PC 80846, Windows 3.11 operating system). The values were normalized to a body surface area of 1.73 m². The ¹²⁵I-labeled iothalamate and ¹³¹I-labeled hippuran concentrations were measured in 3 ml of plasma and urine with a gamma-ray, well-counter spectrometer (COBRA HP; Packard Instrument Co., Downers Grove, Ill.). In this frame, the detected counts on the ¹³¹I energy window were as low as 2 orders of magnitude with respect to the measured counts on the ¹²⁵I energy window. Because of this negligible ratio, the spectrometer standard cross-calibration procedure for depleting the residual influence of the ¹³¹I source on the ¹²⁵I energy window was adopted. The filtration fraction was calculated as the ratio of GFR to ERPF. Renal vascular resistance was calculated by the conventional formula MBP/RBF, where MBP equals mean blood pressure and RBF equals renal blood flow, and was converted from mm Hg/L/min into dyne/sec/cm⁻⁵ by multiplying by 80. Renal blood flow was obtained by using the conventional formula ERPF/(1 - hematocrit).

Urinary prostaglandin assay. PGE₂, PGF_{2α}, 6-keto PGF_{1α}, and TXB₂ were measured in urine because they reflect the renal synthesis of these substances.²⁹ Immediately after collection the urine was frozen and stored at -20° C until extraction and purification procedures could be performed. Urinary samples were extracted by using an organic solvent and then purified by chromatography in a silicic acid column according to the method of Jaffe et al.³⁰ Renal eicosanoids were measured by radioimmunoassay according to the method described by Ciabattini et al.³¹ Professor Carlo Patrono, Istituto di Farmacologia, Università di Chieti, Italy, provided the antibodies to PGE₂ and PGF_{2α}, which were obtained from guinea pigs. Professor Bernard Peskar, Lehrstuhl für Pharmakologie und Toxikologie, Ruhr-Universität, Bochum, Germany, provided the antibody to 6-keto-PGF_{1α}, which was ob-

Table I. Effects of mental stress on plasma catecholamines and plasma renin activity (n = 8)

	Baseline (30 min)	Mental stress (30 min)	Recovery I (30 min)	Recovery II (30 min)	ANOVA for the whole curve	
					F	p
NE (pg/ml)	299.0 ± 191.2	317.5 ± 197.7	261.2 ± 147.5	196.5 ± 114.1	6.66	0.01
Epi (pg/ml)	38.7 ± 8.7	44.0 ± 6.2	33.0 ± 6.3	30.5 ± 5.7	2.9	ns
PRA (ng/ml/hr)	1.62 ± 0.30	1.60 ± 1.00	1.07 ± 0.70	1.13 ± 0.80	5.96	0.01

NE, norepinephrine; Epi, epinephrine.

*p < 0.05 versus baseline, Duncan, ANOVA.

†p < 0.01 versus baseline, Duncan, ANOVA.

Table II. Effects of mental stress on renal hemodynamics (n = 8)

	Baseline (30 min)	Mental stress (30 min)	Recovery I (30 min)	Recovery II (30 min)	ANOVA for the whole curve	
					F	p
ERPF (ml/min/1.73 m ²)	503.7 ± 63.0	395.0 ± 113.0	462.3 ± 50.2	450.1 ± 50.9	3.60	0.03
GFR (ml/min/1.73 m ²)	116.3 ± 20.0	112.2 ± 27.0	129.8 ± 27.3	116.0 ± 25.7	1.03	ns
FF (%)	23.0 ± 4.0	30.0 ± 6.0	28.0 ± 7.0	23.0 ± 5.0	3.53	0.03
RVR (dyne/sec/cm ⁻⁵ /1.73 m ²)	8100 ± 1500	11000 ± 3900	8300 ± 2100	8400 ± 2300	8.50	0.0001

RVR, Renal vascular resistance.

*p < 0.05 versus baseline, Duncan, ANOVA.

†p < 0.02 versus baseline, Duncan, ANOVA.

‡p < 0.001 versus baseline, Duncan, ANOVA.

tained from rabbits. The antibody to TXB₂ obtained from rabbits was provided by Professor Luciano Caprino, Istituto di Igiene, Università Cattolica del Sacro Cuore, Rome, Italy. The sensitivity and cross-reactivities of the antibodies have already been described elsewhere.³¹⁻³³ The average calculated recovery was 53% ± 27% for PGE₂, 58% ± 15% for PGF_{2α}, 56% ± 11% for 6-keto-PGF_{1α}, and 60% ± 10% for TXB₂. The intra-assay and interassay coefficients of variation were 9% and 10%, respectively.

Endothelin assay. The urinary immunoreactive endothelin was measured by radioimmunoassay with a commercially available kit (Endothelin 1/2 RIA system; Biomedica, Vienna, Austria), after having been extracted with C₁₈ SEP-Pack cartridges (Waters-Millipore) according to the method described by Ando et al.³⁴ Because of coextraction of all three isoforms of endothelin and because of the cross-reactivity of the antibody, radioimmunoassay did not discriminate between endothelin-1 and endothelin-2 nor between endothelin-1 and endothelin-3. Nonetheless, because immunoreactive endothelin-1 has been demonstrated to be the predominant isoform in the human kidney,³⁵ the substance that we had measured mostly reflects endothelin-1.

The recovery was 110% ± 7.7% (mean ± SD); the detection limit was 0.8 fmol/ml, and the precision profile showed a coefficient of variation from 25.5% to 6.3% in the 2.5 to 131 fmol/ml range. The within-assay precision was 8.36%, for a mean value of 127 ± 10.7 fmol/ml.

cGMP assay. Nitric oxide production by renal vessels was evaluated by measuring urinary cGMP, which is known to be stimulated by nitric oxide and to mediate nitric oxide action in the kidney.^{19,21} Urine samples were stored at -20° C until assay. cGMP was directly measured in defrosted urinary samples after dilution in assay buffer, without any preliminary extraction. Urinary cGMP was measured by radioimmunoassay with a commercially available kit (cGMP-[¹²⁵I] assay system RPA 525; Amersham Laboratories, Buckinghamshire, England) with a nonacetylation technique according to the method described by Steiner et al.³⁶ The standard curve ranged from 50 to 6400 fmol/tube.

The values of the urinary substances, measured during all of the experimental periods, were normalized for individual baseline ERPF, which was expressed in ml/min/1.73 m², as an index of renal mass.

Plasma renin activity. PRA was determined by radioimmunoassay with a commercially available kit (Angio-

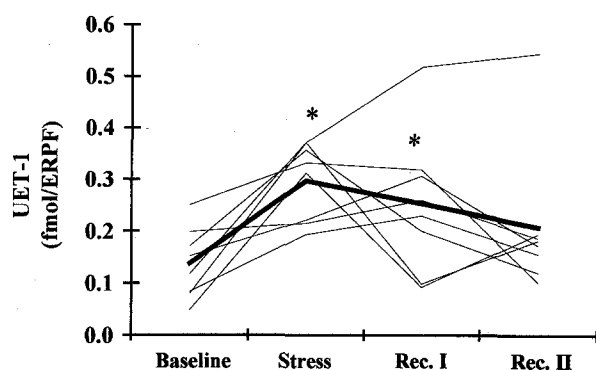


Fig. 3. Effects of mental stress on urinary excretion of endothelin (UET-1) ($n = 8$). ERPF is expressed in ml/min/1.73 m². *Rec I*, Recovery period I; *Rec II*, recovery period II. * $p < 0.01$ vs baseline, Duncan test, ANOVA. Thin lines indicate individual data; thick line indicates mean values.

tensin RIA CT; RADIM, Rome, Italy) with the method described by Haber et al.³⁷

All radioimmunoassays were performed when the radioactivity, because of the ¹²⁵I and ¹³¹I used for clearance, had dropped to low enough values in the collected urine and plasma so as not to interfere with the radioimmunoassay. This drop was verified by counting periodically the radioactive levels in a sample that had been taken from each subject and stored for this purpose. To ensure that the level of radioactivity was low enough to avoid interference, a 3-month period was usually necessary.

Furthermore, to validate our methods and to rule out any possible influence of a residual trace amount of radioactivity, we included blank tubes (all reagents except the first antibody) in all standard and sample series for correction of standard and sample counts, respectively. We did not find any significant differences ($p = 0.7$) between the blank tubes of the standard series and the blank sample tubes.

Catecholamine assay. Plasma catecholamines were determined by electrochemical detection according to the method described by Mefford et al.³⁸ The calculated recovery determined by measuring the final concentration of the internal standard was 89 ± 5.8 for norepinephrine and 90.2 ± 3.1 for epinephrine. In our laboratory the normal ranges of plasma norepinephrine and epinephrine concentrations are 130 to 400 pg/ml and 25 to 100 pg/ml, respectively. The limit of detection was 10 pg/ml for norepinephrine and epinephrine. The intra-assay coefficient of variation was 4.3% for norepinephrine and 3.1% for epinephrine. The interassay coefficient of variation was 7.9% for norepinephrine and 6.5% for epinephrine. The concentration of tritiated norepinephrine was determined by liquid scintillation counter after extraction with alumina.

Urinary electrolytes. Urinary electrolytes were measured by using a flame photometer (system 243; Instrumentation Laboratory, Lexington, Mass.).

Statistical analysis. All results have been presented as mean \pm SD. One-way ANOVA and Duncan's test were used

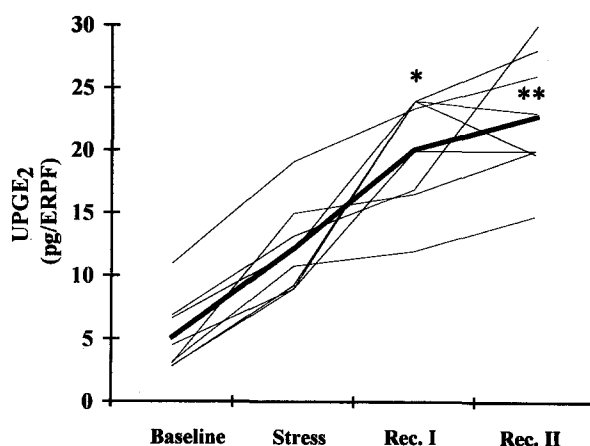


Fig. 4. Effects of mental stress on UPGE₂ ($n = 8$). ERPF is expressed in ml/min/1.73 m². *Rec I*, Recovery period I; *Rec II*, recovery period II. * $p < 0.01$, ** $p < 0.001$ vs baseline, Duncan test, ANOVA. Thin lines indicate individual data; thick line indicates mean values.

to study the effects induced by mental stress on all parameters. Linear regression analysis was performed by the least square method. The level of probability was set at 0.05.

RESULTS

Mental stress induced significant changes in blood pressure and HR that were limited to the period of administration of the stimulus (Fig. 2). Systolic blood pressure increased from a baseline value of 114.4 ± 14.0 mm Hg to a peak of 134.2 ± 17.2 mm Hg (+17%, $p < 0.001$); diastolic blood pressure increased from 76.0 ± 7.0 mm Hg to 98.0 ± 0.3 mm Hg (+28%, $p < 0.001$), and HR increased from 74.0 ± 13.0 beats/min to 86.9 ± 14.0 beats/min (+17%, $p < 0.001$) (Fig. 2).

Plasma norepinephrine increased slightly but significantly during mental stress (317.5 ± 197.7 pg/ml, vs a baseline value of 299 ± 191.2 pg/ml, $p < 0.05$) and then dropped below baseline values, reaching a minimum value during the second recovery period (196.5 ± 114.1 pg/ml, $p < 0.01$ vs baseline) (Table I). Plasma epinephrine did not significantly change during the entire study period. Plasma renin activity did not change during mental stress but showed a slight-though-significant decrease during the recovery periods (1.07 ± 0.7 ng/ml/hr during the first recovery period and 1.13 ± 0.7 ng/ml/hr during the second recovery period, vs 1.62 ± 0.3 ng/ml/hr during baseline, $p < 0.01$; $p < 0.01$ with Duncan ANOVA) (Table I).

Mental stress was also associated with relevant changes in renal hemodynamics. With the exception of GFR, all of the variables were modified as

Table III. Effects of mental stress on urinary volume and sodium excretion (n = 8)

	Baseline (30 min)	Mental stress (30 min)	Recovery I (30 min)	Recovery II (30 min)	ANOVA for the whole curve	
					F	p
UV (ml/30 min)	137.1 ± 100.8	63.7 ± 37.0	71.8 ± 55.6	58.0 ± 37.1	1.13	ns
UNa ⁺ (mmol/30 min)	14.4 ± 11.7	11.2 ± 2.3	7.2 ± 3.2	10.4 ± 3.4	1.76	ns
FE _{Na} (%)	14.4 ± 16.6	4.7 ± 3.8	2.9 ± 2.1	4.0 ± 3.3	2.60	ns

UV, Urinary volume; UNa⁺, urinary sodium; FE_{Na}, fractional excretion of sodium.

*p < 0.05 versus baseline, Duncan, ANOVA.

†p < 0.02 versus baseline, Duncan, ANOVA.

Table IV. Effects of mental stress on urinary autacoids (n = 8)

	Baseline (30 min)	Mental stress (30 min)	Recovery I (30 min)	Recovery II (30 min)	ANOVA for the whole curve	
					F	p
6-keto-PGF _{1α} (pg/ERPF)	12.0 ± 0.1	15.0 ± 6.0	17.0 ± 8.0	15.0 ± 4.0	6.71	0.001
PGF _{2α} (pg/ERPF)	42.0 ± 14.0	58.0 ± 18.0	60.0 ± 14.0	49.0 ± 14.0	1.65	ns
TXB ₂ (pg/ERPF)	7.2 ± 2.8	15.0 ± 9.0	11.9 ± 9.0	7.0 ± 4.2	2.11	ns
6-keto-PGF _{1α} /TXB ₂ ratio	1.8 ± 0.4	1.6 ± 0.3	3.4 ± 1.9	5.0 ± 1.8	12.70	0.001
PGE ₂ /PGF _{2α} ratio	0.13 ± 0.06	0.19 ± 0.10	0.35 ± 0.20	0.43 ± 0.09	8.30	0.01
cGMP (pg/ERPF)	40.4 ± 21.4	71.8 ± 39.9	36.8 ± 16.1	52.3 ± 25.7	4.13	0.01

ERPF is expressed as ml/min/1.73 m².

*p < 0.01 versus baseline, Duncan, ANOVA.

†p < 0.001 versus baseline, Duncan, ANOVA.

demonstrated by ANOVA for the whole curve (Table II). ERPF decreased significantly during mental stress (−22%, $p < 0.05$) and then reverted to baseline values. The GFR, on the contrary, did not vary throughout the four experimental periods. A significant increase in filtration fraction occurred during mental stress and the first recovery period (+30%, $p < 0.02$, and +22%, $p < 0.02$, vs baseline). Renal vascular resistance increased during mental stress (+37%, $p < 0.001$).

Although mental stress had minor effects on urinary volume, fractional sodium excretion significantly decreased during the mental stress and recovery periods (Table III). Among the urinary autacoids, mental stress induced a significant increase in endothelin ($p < 0.01$) and PGE₂ ($p < 0.001$), as shown by ANOVA for the whole curve (Figs. 3 and 4). Urinary endothelin-1 increased during mental stress and during the first recovery period ($p < 0.01$ for both) when compared

with baseline values by using Duncan ANOVA (Fig. 3). UPGE₂ increased with growing intensity up to the second recovery period, when it reached a peak value that was four times higher than baseline (Fig. 4). U6-keto-PGF_{1α} increased during mental stress and the two recovery periods ($p < 0.01$); UPGF_{2α} and UTXB₂ did not significantly increase. U6-keto-PGF_{1α}/UTXB₂ and UPGE₂/UPGF_{2α} ratios increased during the two recovery periods ($p < 0.01$ and $p < 0.001$, respectively, during both experimental periods) (Table IV).

cGMP rose only during the stimulus (71.8 ± 39.9 pmol/ERPF during mental stress vs 40.4 ± 21.4 pmol/ERPF; $p < 0.05$) and subsequently dropped to baseline value during the first recovery period (Table IV).

DISCUSSION

The mental stress that we used induced a significant and sustained increase in plasma norepinephrine, HR, and both systolic and diastolic blood pres-

sure associated with a 20% reduction in ERPF and a constant GFR, effects characteristic of those produced by activation of the sympathetic nervous system.^{1,2} The trend toward water retention that occurred was associated with a significant decrease in fractional sodium excretion. These findings are also consistent with increased renal adrenergic activity.³⁹ We did not obtain any evidence of systemic activation of the renin angiotensin system given that PRA did not increase.

Changes in the renal hormonal profile having different time courses were seen in the excretory patterns of prostaglandins, endothelin-1, and cGMP. Activation of these hormonal systems resulted from the initial sympathetic stimulation followed by interactions among involved hormones.³⁻⁵ For example, increased renal sympathetic activity enhances endothelin-1 and prostaglandin release, chiefly that of PGE₂, as its primary effects.^{4,10} Endothelin-1, in turn, can act as an additional stimulus to PGE₂ formation.¹¹ Thus the progressive increase in excretion of PGE₂ for 60 minutes beyond the cessation of the stimulus of mental stress may be attributed to stimulation by endothelin-1 as well as promotion of renal prostaglandin synthesis by the initial increase in renal adrenergic activity.⁴ A close link between endothelin and PGE₂ has been described in rats and dogs after pharmacologic stimulation.^{11,12} The relatively selective involvement of PGE₂ is in keeping with the well-defined modulating action of this eicosanoid on pressor systems.^{4,40} It also should be noted that urinary endothelin excretion may reflect events separate from those expressed by plasma endothelin levels, because there is evidence that plasma and renal endothelin represent distinct systems.^{34,41}

The several components of the neurohumoral response to mental stress may have differential effects on GFR. For example, PGI₂ and PGE₂ have been reported to produce prevalent dilation of the afferent arteriole,⁴⁰ whereas endothelin and angiotensin II have more prominent effects on the efferent arteriole.^{3,42} Even if peripheral venous PRA was not changed by the stimulation in the present investigation, these data cannot rule out a possible role of renal tissue angiotensin II in the adaptation to stress. Indeed, the decrease in fractional sodium excretion during mental stress that was observed in our study can support the hypothesis of an increase in tissue angiotensin II. In addition, adrenergic stimulation and reduced renal perfusion are known to increase intrarenal angiotensin II production.^{3,43}

The present study does not allow assignment of weight to these hormonal inputs that presumably contribute to the regulation of GFR, because this would require an experimental preparation that allowed con-

trol of important variables such as isolated perfused glomeruli.

We have shown an early increase in the excretion of cGMP that reflects the renal production of nitric oxide or ANF.^{21,22} Because we did not measure ANF, only tentative conclusions can be reached concerning the relationship of cGMP to renal nitric oxide production. However, under these experimental conditions of constant circulating volume, stimulation of ANF is not likely.⁴⁴ In addition, stress-induced adrenergic activation probably does not result in ANF production, because there is evidence that in the face of increased sympathetic stimulation, ANF release is either blunted or unchanged.^{45,46}

In conclusion, the renal response to adrenergic stimulation induced by mental stress is a complex phenomenon that involves the interaction of several neurohumoral systems. Endothelin participates in renal homeostatic mechanisms together with prostaglandins and possibly nitric oxide. Furthermore, these factors have different time courses and can be expected to influence the biologic activity of each other as well as the overall duration of the activity. These time relationships indicate the renal vasoconstrictive role of endothelin that is modulated by renal vasodilative autoacids, in particular PGE₂.

We thank Professor John C. McGiff, Department of Pharmacology, New York Medical College, Valhalla, New York, and Professor Quiermo Maggiore, Ospedale Santa Maria Annunziata, Firenze, Italy, for helpful suggestions and assistance in preparing the manuscript. We also thank Professor Carlo Patrono, Istituto di Farmacologia, Università di Chieti, Italy, for the gift of PGE₂ and PGF_{2α} antisera; Professor Bernard Peskar, Department of Pharmacology, Bochum University, Germany, for providing the antiserum to 6-keto-PGF_{1α}; and Professor Luciano Caprino, Istituto di Igiene, Università Cattolica del Sacro Cuore, Roma, Italy, for providing the TXB₂ antiserum. In addition, we thank Dr. Alessandro Passeri for assistance in the computerized calculation of renal hemodynamic parameters.

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